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New records and range expansion for *Paspalum procurrens* and *P. volcanense* in northwestern Argentina and southeastern Bolivia

Adriana Glücksberg[1], Eric Javier Martínez[1], Ana Isabel Honfi[2], Carla Maldonado[3], Diego Hernán Hojsgaard[4]

1 Instituto de Botánica del Nordeste (IBONE, CONICET-UNNE), Calle Sargento Cabral 2131, CP 3400, Corrientes Capital, Corrientes, Argentina. **2** Programa de Estudios Florísticos y Genética Vegetal (IBS, CONICET-UNaM) nodo Posadas, Calle Rivadavia, 2370, CP 3300, Posadas, Misiones, Argentina. **3** Herbario Nacional de Bolivia, Universidad Mayor San Andrés, c/ N° 27 Cota Cota, Campus Universitario UMSA, Casilla 1007 Correo Central, La Paz, Bolivia. **4** Department of Systematic, Biodiversity and Evolution of Plants (with Herbarium), Albrecht-von-Haller Institute for Plant Science, Georg-August-University of Goettingen, Untere Karspuele 237073, Goettingen, Germany.
Corresponding authors: Adriana Glücksberg, adriglucks@gmail.com; Diego Hernán Hojsgaard, dhojsga@gwdg.de

Abstract

Paspalum procurrens Quarin and *P. volcanense* Zuloaga, Morrone & Denham are two rare species of South American grasses inhabiting geographically restricted areas which are exposed to ecological degradation due to landscape transformation and biodiversity losses. We present new records for these species, from the provinces of Jujuy, Salta, Tucumán and Catamarca (Argentina) and departments of Tarija, Chuquisaca, and Santa Cruz (Bolivia). New geographic distribution maps and the biological relevance of these findings are discussed pinpointing the need for imperative and assiduous botanical explorations in biodiversity hotspots sensible to the impact of human activities. Ploidy levels in these two species are studied.

Keywords

Anachyris, biodiversity, grasses, rare species, South America, polyploidy.

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Introduction

Worldwide, human activities cause major changes in biological communities which threaten biodiversity, ecosystem function, and human well-being (Díaz et al. 2006). The main threats facing biodiversity are related to changes in land cover, habitat degradation and fragmentation, exploitation of natural resources, and introduction of alien species. Studies have noted that species respond to these threats in varying ways, often by showing phylogenetic and ecological patterns; therefore, there is a need to model how biodiversity responds to threats of different intensity in association with diverse ecological attributes of species (Díaz et al. 2013). With this in mind, cataloguing biodiversity is critically needed to provide biodiversity measures, and botanical expeditions and botanical gardens play a central role in the study and conservation of plants; they reveal the world's diversity of plant species and contribute to human needs and human well-being (Bleichmar 2007; Chen and Weibang 2018).

The genus *Paspalum* L. comprises 310 species (Morrone et al. 2012) living in a wide variety of habitats and constitutes an important part of the vegetation particularly in open grasslands (Zuloaga and Morrone 2005).

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The most common habitats for these species include savannahs and forest borders (Morrone et al. 2000). *Paspalum* is one of the most economically important genera within the tribe Paniceae, because its species produce high-quality forage, have ornamental value, and are used as food or in folk medicine (Zuloaga and Morrone 2005). Furthermore, considering the ecological amplitude found among species, and the reproductive, morphological, and genetic variability present in the group (Zuloaga and Morrone 2005), *Paspalum* is of scientific interest for a variety of studies, including topics on evolution, like the origin and distribution of new apomicts (e.g. Hojsgaard 2018) and applied agronomy, like breeding of native forages (Marcón et al. 2018; Zilli et al. 2018, 2019). Many *Paspalum* species are characterized by the existence of conspecific cytotypes, i.e. different individuals of the same species carrying diverse ploidy levels. The basic chromosome number is generally $x = 10$, and *Paspalum* species exhibit cytotypes ranging from diploids ($2n = 2x = 20$ chromosomes) to hexadecaploids ($2n = 16x = 160$ chromosomes), with tetraploids being the most frequent condition (Hojsgaard et al. 2009). The genus *Paspalum* is subdivided in four subgenera (Denham 2005): 1) *Anachyris* (Nees) Chase, a monophyletic group of six species sharing genetic, embryological, and morphological features (Morrone et al. 2000; Urbani et al. 2002; Hojsgaard 2010; Rua et al. 2010; Zilli et al. 2014); 2) *Ceresia* (Pers.) Rcbh., a group of 25 species characterized by having winged rachises (Denham et al. 2002); 3) *Harpostachis* (Trin.) S. Denham, a grouping 40 species mainly from Central America and northern South America, characterized by having solitary racemes, terminal and axillary inflorescences in the upper foliar sheaths, lower glumes, and a staminate lower flower (Denham 2005; Sánchez-Ken 2010); and 4) *Paspalum* sensu stricto, with the most species (around 300) and the greatest morphological diversity (Zuloaga and Morrone 2005).

Anachyris is the smallest subgenus within *Paspalum*, comprising *P. costellatum* Swallen, *P. malacophyllum* Trin., *P. procurrens* Quarin, *P. simplex* Morong, *P. usterii* Hack., and *P. volcanense* Zuloaga, Morrone & Denham. All species are restricted to South America, except for *P. malacophyllum,* which occurs north into Mexico. Other features of *Anachyris* species are the perennial habit (except the annual *P. costellatum*), caespitose (except the stoloniferous *P. procurrens*), the absence of navicular spikelets with the lower and the upper glumes (occasionally present in *P. volcanense* and in *P. usterii*), and the lower and the upper lemma markedly ribbed (Quarin 1993; Morrone et al. 2000; Zuloaga et al. 2000). While *P. simplex* and *P. malacophyllum* are the most common species in terms of frequency and distribution, all other four species are rare, displaying rather restricted distributions, associated with specific ecological habitats.

Paspalum procurrens inhabits northwestern Argentina and southeastern of Bolivia. This species lives in sandy and rocky soils (Cabrera 1994), in the semi-arid Salta Chaco and in the Chaco forests of southeastern Bolivia, from sea level to 800 m a.s.l. (Morrone et al. 2000; Zuloaga and Morrone 2005). In this species, diploid (Quarin 1993) and tetraploid (Hojsgaard et al. 2008; Zilli et al. 2014) cytotypes have been reported. *Paspalum volcanense* has been found in the province of Jujuy (Argentina) and in the Department of Tarija (in Bolivia), between 1000–2300 m a.s.l. It grows in permeable, immature, rocky and sandy soils in the Yungas (Cabrera 1994) and in transition areas between the Yungas and the Prepuneña province (Morrone et al. 2000; Zuloaga and Morrone 2005). This species was reported to be tetraploid (Hunziker et al. 1998; Hojsgaard et al. 2008; Zilli et al. 2014).

In this work, we present new records for *P. procurrens* and *P. volcanense* found during a botanical expedition in areas associated with the known habitats and geographic distributions of these species. We also evaluate the ploidy diversity of the new occurrences and discuss the importance of botanical collections in South American biodiversity hotspots subjected to great habitat loss.

Methods

Collection of plant materials and species identification. Samples of *Paspalum procurrens* and *P. volcanense* were collected on 3–11 March 2018, during the flowering season, which is the only period when it is possible to distinguish these species from among other grasses by their reproductive characters. The area of botanical study was in northwestern Argentina in the provinces of Catamarca, Chaco, Jujuy, Salta, northern Santiago del Estero, and Tucumán, and southeastern Bolivia in the departments of Tarija, Chuquisaca, and Santa Cruz.

Both species were brought to the experimental gardens at the Facultad de Ciencias Agrarias of the Universidad Nacional del Nordeste (Corrientes, Argentina) for cultivation. Vouchers were prepared and deposited in the Herbario Nacional de Bolivia (LPB), the herbarium of the Universidad Nacional de Misiones (MNES), and the herbarium of the Instituto de Botánica del Nordeste (CTES) (acronyms according to Thiers 2019).

The individuals of *P. procurrens* Quarin and *P. volcanense* Zuloaga, Morrone & Denham were first identified while in the field by their habit of growth and inflorescences; they were differentiated from other native southern South American *Paspalum* with the help of a taxonomic key (Zuloaga and Morrone 2005). Later, herborized specimens and other preserved field material (e.g. inflorescences) were examined in the lab under a stereomicroscope to identify diagnostic morphological characters (mainly from spikelets) and corroborate our initial species identitifications.

Preservation, processing, and storage of specimens. The identity of all specimens were confirmed through specialized literature on the subgenus *Anachyris* (Mor-

rone et al. 2000). For each species and locality point, at least three plant specimens were collected, processed *in situ*, and assigned a single collection number (British Columbia Ministry of Forests 1996; Maden 2004), which were then kept in the plant press throughout the field trip, and once back at the Institute they were dried at 60–75 °C using a forced-air heat vent, labeled, and mounted on herbarium sheets for preservation.

Species distribution analysis. A map showing the geographic distribution of each species was prepared using ArcGis 10.5.1 (ESRI, 2017) and data from SpeciesLink (http://www.splink.org.br), the Global Biodiversity Information Facility (http://www.gbif.org), and data from CTES and of our own collections. Additional information on species occurrences was collected from the literature and from material examined at nine herbaria (B, BAA, GH, GOET, LPB, MNES, MO, PE and SI; see details in Table 1; acronyms follow Thiers 2019). When the geographic coordinates of the collection sites were not explicit, coordinates were subsequently assigned using the nearest locality point. Information about terrestrial ecoregions was obtained from the Terrestrial Ecoregions of the World (http://www.worldwildlife.org; Olson 2001). We followed Gibson's (2009) definition of ecoregions as fine-scale regional ecological areas within a biome characterized by local geographic and climatic conditions and unique assemblages of species.

Ploidy analyses. The ploidy level of each individual was determined by Flow Cytometry (FC) following the methodology described by Sartor et al. (2011). Briefly, ca 0.5 cm^2 leaf material of each sample was placed in a glass Petri dish, 0.5 ml of extraction buffer was added, and the sample chopped with a sharp razor blade. After a 2-min incubation time, samples were filtered through a 50 µm nylon mesh directly into the sample tube and 1.5 ml DAPI (4′,6-diamidino-2-phenylindole) stain solution (Partec P kit CySatin UV) was added. The mixture was incubated for 2 min at room temperature and analyzed with a Partec PA II flow cytometer (Sysmex-Partec GmbH, Münster, Germany). Histograms with a

Table 1. List of *Paspalum procurrens* and *P. volcanense* specimens included in this study.

Localities	Coordinates	Date	Voucher	Ploidy	
				2n	Reference
Paspalum procurrens					
Argentina, Salta, Anta	25°13′26″S, 064°05′22″W	01/04/1971	Saravia Toledo et Parada 45 (CTES)	ND	
Argentina, Salta, Anta	25°12′45″S, 064°05′43″W	10/04/1974	Cuezzo 10104 (CTES)	ND	
Argentina, Salta, Metán	25°31′38″S, 064°58′36″W	28/03/1975	Krapovickas et al. 27920 (CTES)	ND	
Argentina, Salta, Anta	25°13′08″S, 064°05′29″W	30/01/1984	Saravia Toledo 822 (CTES)	ND	
Argentina, Salta, Anta	24°45′03″S, 063°54′46″W §	30/03/1985	Del Castillo et Varela 713 (CTES)	ND	
Argentina, Salta, Anta	25°12′54″S, 064°02′03″W	15/03/1986	Saravia Toledo 1195 (CTES, SI)	ND	
Argentina, Salta, Anta	25°12′33″S, 064°03′30″W	20/02/1987	Saravia Toledo 1331 (CTES, SI)	ND	
Argentina, Salta, Anta	24°48′36″S, 063°49′48″W	14/04/1989	Saravia Toledo 2068 (BAA, CTES, K, SI, US)	ND	
Argentina, Salta, Anta	25°06′00″S, 064°06′00″W ‡	26/03/1991	Quarin 4060 (BAA, CTES, K, MBM, MO, SI, UB, US)	20	Quarin 1993
Argentina, Salta, General Güemes, La Trampa	24°52′50″S, 064°50′34″W ‡	21/02/1992	Saravia Toledo 10685 (CTES)	ND	
Argentina, Salta, Guachipas	25°30′05″S, 065°31′42″W ‡	22/03/1996	Saravia Toledo 13845 (CTES)	ND	
Argentina, Salta, Anta	25°14′04″S, 064°23′43″W	18/12/2005	Hojsgaard 373 (CTES, ICN)	40	Hojsgaard 2010; Zilli et al. 2014
Argentina, Salta, Anta	25°19′03″S, 064°01′06″W	19/12/2005	Hojsgaard 376 (CTES, CEN)	40	Hojsgaard 2010; Zilli et al. 2014
Argentina, Salta, Lumbrera	25°11′54″S, 064°53′38″W	07/03/2018	Martínez et al. 80 (CTES)	20	This work
Bolivia, Chuquisaca, Luis Calvo	19°05′38″S, 065°17′54″W ‡	04/06/1991	Saravia Toledo 2935 (CTES)	ND	
Bolivia, Chuquisaca, Luis Calvo	20°34′45″S, 063°17′39″W ‡	23/03/1992	Saravia Toledo 10862 (MO)	ND	
Bolivia, Chuquisaca, Luis Calvo	20°28′54″S, 063°16′45″W ‡	26/03/1993	Quarin 4094 (CTES, LPB, MO)	40	Hojsgaard et al. 2008
Bolivia, La Paz, Bautista Saaveedra	15°11′20″S, 068°48′36″W ‡	21/04/1994	Feuerer 6213 (K, MO)	ND	
Bolivia, Chuquisaca, Luis Calvo	20°34′45″S, 063°17′39″W ‡	17/02/1995	Saravia Toledo 12646 (CTES)	ND	
Bolivia, Chuquisaca, Luis Calvo	20°34′45″S, 063°17′39″W ‡	12/03/1996	SaraviaToledo 13818 (CTES)	ND	
Bolivia, Chuquisaca, Machareti	20°46′24″S, 063°18′57″W	11/03/2018	Martínez et al. 91 (CTES, LPB, MNES)	40	This work
Bolivia, Santa Cruz, La Herradura	19°30′13″S, 063°32′48″W	11/03/2018	Martínez et al. 96 (CTES, LPB, MNES)	20, 40	This work
Paspalum volcanense					
Argentina, Jujuy, Tumbaya	23°54′00″S, 065°27′00″W ‡	ND	Morrone 4374 (CTES, MO, SI)	40	Hojsgaard 2010; Zilli et al. 2014
Argentina, Jujuy, Tumbaya	23°56′00″S, 065°27′00″W ‡	06/03/1965	Cabrera et Solbrig 16850 (GH)	ND	
Argentina, Jujuy, Tumbaya	23°56′00″S, 065°27′00″W ‡	13/02/1985	Kiesling et al. 5170 (MO, SI, US)	ND	
Argentina, Jujuy, Tumbaya	23°56′00″S, 065°27′00″W ‡	13/02/1985	Kiesling et al. 5171 (MO, SI)	ND	
Argentina, Jujuy, Tumbaya	23°56′00″S, 065°27′00″W	15/02/1997	Zuloaga et al. 5871 (CTES, MO, SI)	ND	
Argentina, Jujuy, Tumbaya	23°56′39″S, 065°27′05″W	17/12/2005	Hojsgaard 370 (CEN, CTES, GH, ICN)	40	Hojsgaard 2010; Zilli et al. 2014
Argentina, Catamarca, El Rodeo	28°18′31″S, 065°53′53″W	05/03/2018	Martínez et al. 55 (CTES, MNES)	ND	
Argentina, Tucumán, El Rincón	26°57′12″S, 065°46′49″W	05/03/2018	Martínez et al. 68 (no voucher specimen collected)	ND	
Argentina , Jujuy, Tumbaya	23°56′39″S, 065°27′09″W	06/03/2018	Martínez et al. 77 (CTES)	40	This work
Bolivia, Tarija, Méndez	21°32′00″S, 064°50′00″W	10/05/1983	Solomon 10630 (MO)	ND	
Bolivia, Tarija, Junacas	21°25′28″S, 064°24′27″W	10/03/2018	Martínez et al. 88 (CTES, LPB; MNES)	40	This work

ND: No data. ‡Geographic coordinates subsequently assigned. §The geographic center of Anta Department was used.

relative fluorescence intensity of >3000 nuclei and maximum coefficient of variation (CV) values of 15% were accepted for each sample peak (G_0/G_1 peak). Ploidy levels were estimated by comparing mean values of each sample peak to an internal control sample (a plant of the same species with known chromosome number). For *P. procurrens* the plant genotype Hojsgaard 373 ($2n = 2x = 40$) was used as control, and for *P. volcanense* the genotype Morrone 4374 ($2n = 2x = 40$) was used as control (Zilli et al. 2014). In a few cases, the FC ploidy level was verified by chromosome counting according to the Feulgen method (Honfi et al. 1990), using mitotic metaphase cells observed under a Leica DM5500B microscope (Leica Microsystems GmbH, Wetzlar, Germany).

Results

Table 1 shows the data available for each of the species studied, including previous collections from herbaria and those obtained for this work. The data underpinning the analysis reported in this paper was deposited at GBIF, the Global Biodiversity Information Facility, and are available at (https://doi.org/10.15468/csjzsx).

Paspalum procurrens

New records. ARGENTINA • Martínez et al. 80 (CTES); Salta, Lumbrera, next to Provincial Route 5; 25°11′54″S, 064°53′38″W ± 25–30 m; alt. ca 730 m; 7 Mar. 2018.

BOLIVIA • Martínez et al. 91 (CTES, LPB, MNES); Chuquisaca, Machareti, next to Provincial Route 9; 20°46′24″S, 063°18′58″W ± 25–30 m; alt. ca 650 m; 11 Mar. 2018. • Martínez et al. 96 (CTES, LPB, MNES); Santa Cruz, La Herradura, next to Provincial Route 9; 19°30′13″S, 063°32′48″W ± 25–30 m; alt. ca 880 m; 11 Mar. 2018.

Taxonomic treatment. *Paspalum procurrens* plants had concave-convex spikelets, the upper lemma with abaxial side furrowed, nerves marked, glabrous spikelets, and with the upper glume absent. This species was easily distinguished from others species within the subgenus because it is the only stoloniferous species within *Anachyris* (Fig. 1a, b).

Paspalum volcanense

New plant records: ARGENTINA • Martínez et al. 55 (CTES; MNES); Catamarca, El Rodeo, next to Provincial Route 4; 28°18′31″S, 065°53′53″W ± 25–30 m; alt. ca 1570 m; 4 Mar. 2018. • Martínez et al. 68 (voucher specimen not collected); Tucumán, El Rincón, close to the River El Mollar, next to Provincial Route 325; 26°57′12″S, 065°46′49″W ± 25–30 m; alt. ca 2360 m; 5 Mar. 2018. • Martínez et al. 77 (CTES); Jujuy, Tumbaya, quarry of Volcán, next to the Río Grande; 23°56′39″S, 065°27′09″W ± 25–30 m; alt. ca 2015 m; 6 Mar. 2018.

BOLIVIA, Tarija • Martínez et al. 88 (CTES, LPB, MNES); Junacas, next to National Route 11; 21°25′28″S, 64°24′27″W ± 25–30 m; alt. ca 2470 m; 10 Mar. 2018.

Taxonomic treatment. *Paspalum volcanense* is distinguished by its concave-convex spikelets, the upper lemma with abaxial side furrowed with marked ribs, and hairy spikelets in which the upper glume is mostly present (ranging from rudimentary to well developed, in some cases reaching the full length of the spikelet). The collected plants ranged from 30 cm tall (those in vegetative state) to around 130 cm (those having flowering stems), the rhizomes had short internodes and reeds were herbaceous (Fig. 1c, d).

Species distribution, habitats and ploidy levels. The areas in Bolivia where the samples were collected correspond mainly to the Dry Chaco ecoregion, and in Argentina they were obtained from the Southern Andean Yungas and Dry Chaco ecoregions (Fig. 2). The specimens of *P. volcanense* were collected from rocky-soil habitats, on cliffs, between 1500 and 2500 m a.s.l, and the samples of *P. procurrens* were collected in sandy and stony habitats, between 600 and 1000 m a.s.l.

Our flow cytometry analyses of ploidy in populations of *P. procurrens* displayed ploidy variability. Two collection sites showed constancy in ploidy levels between individuals within populations, but they differed among populations (population Martínez et al. 80 was diploid, $2n = 2x = 20$; population Martínez et al. 91 was tetraploid $2n = 4x = 40$), the population of a third collection site (Martínez et al. 96) presented diploid and tetraploid individuals. In *P. volcanense*, all individuals analyzed from the populations Martínez et al. 77 and Martínez et al. 88 were tetraploid ($2n = 4x = 40$; Figure 3a). Chromosome counts in five individuals corroborated ploidy levels inferred from FC (Fig. 3b).

Discussion

Meadows and grasslands have played an important role for humans since the beginning of humanity. Human exploitation and transformation of grasslands into pastures has brought ecological changes in this biome which normally sustains a high diversity of plant and animal species, and more recently, large worldwide losses in land cover (see e.g. Haines-Young and Potschin 2010). The major modifications in natural grasslands are from agriculture, landscape fragmentation, invasion from non-native species, fire or lack of fire, desertification, human settlements and urbanization, and domestic livestock (Gibson 2009). In the areas visited in this study, biodiversity of local meadows is affected by new infrastructures such as road construction, as well as the expansion of agricultural land and livestock areas.

Undoubtedly, roads and agriculture have contributed the most to the loss and fragmentation of high-elevation grasslands. Particularly in Bolivia, almost 50% of the national territory is considered an area with hydrocarbon potential, and the oil industry together with agricultural activities have had an important role in the loss of biodiversity (Ibisch and Mérida 2003). This is especially

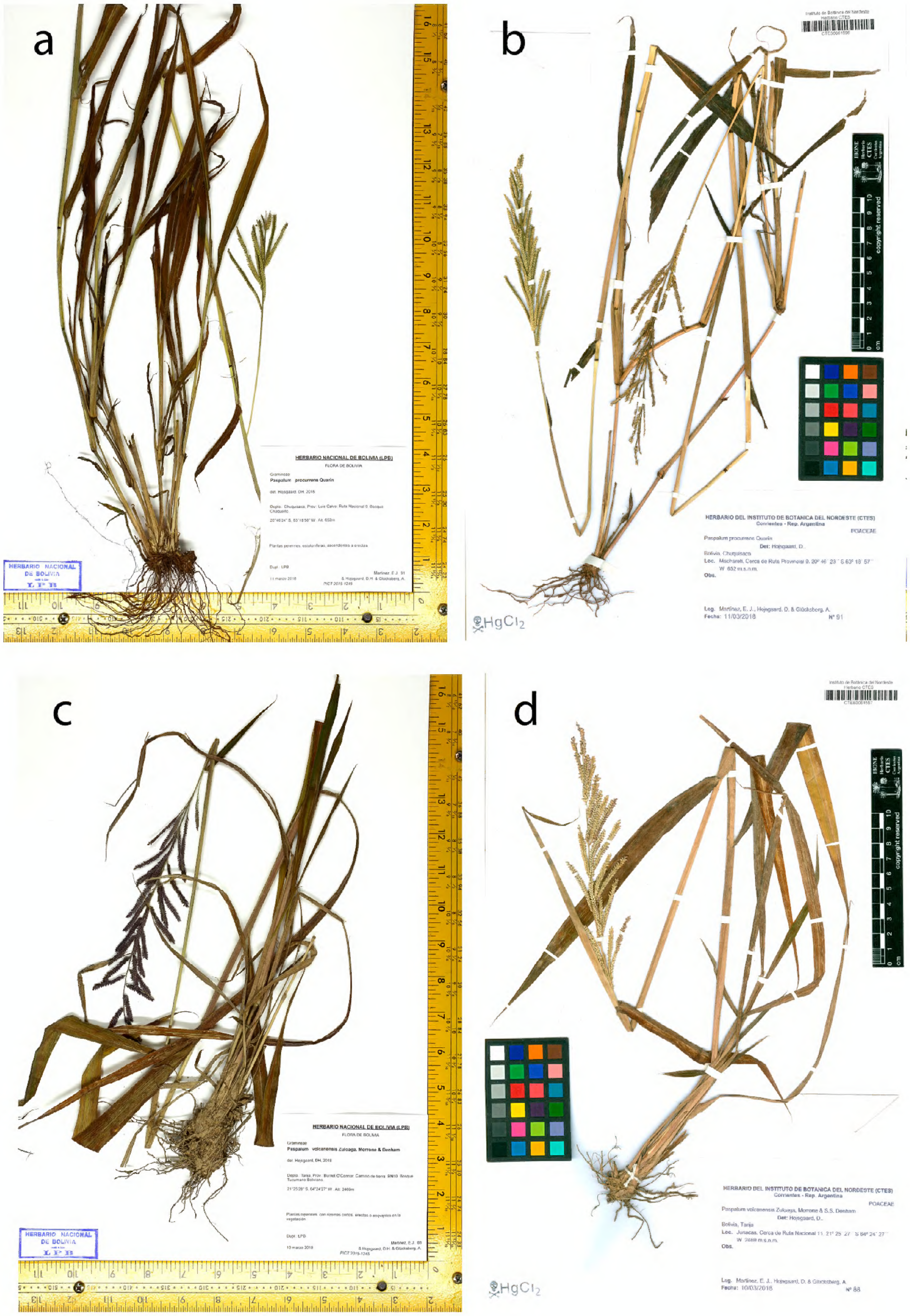
a
HERBARIO NACIONAL DE BOLIVIA (LPB)
FLORA DE BOLIVIA
Gramineae
Paspalum procurrens Quarin
det. Hojsgaard, DH. 2018
Depto. Chuquisaca, Prov.: Luis Calvo. Ruta Nacional 9. Bosque Chaqueño.
20°46'24" S, 63°18'58" W. Alt. 652m
Plantas perennes, estoloníferas, ascendentes a erectas
Dupl.: LPB
11 marzo 2018
Martinez, E.J. 91
& Hojsgaard, D.H. & Glücksberg, A.
INCT 2015-1245
HERBARIO NACIONAL DE BOLIVIA
LPB
b
HERBARIO DEL INSTITUTO DE BOTANICA DEL NORDESTE (CTES)
Corrientes - Rep. Argentina
POACEAE
Paspalum procurrens Quarin
Det: Hojsgaard, D.
Bolivia. Chuquisaca
Loc. Machareti, Cerca de Ruta Provincial 9. 20° 46' 23" S 63° 18' 57" W 652 m.s.n.m.
Obs.
Leg. Martínez, E. J., Hojsgaard, D. & Glücksberg, A.
Fecha: 11/03/2018
N° 91
HgCl2
c
HERBARIO NACIONAL DE BOLIVIA (LPB)
FLORA DE BOLIVIA
Gramineae
Paspalum volcanensis Zuloaga, Morrone & Denham
det. Hojsgaard, DH. 2018
Depto. Tarija, Prov.: Burnet O'Connor. Camino de tierra RN10 Bosque Tucumano Boliviano.
21°25'28" S, 64°24'27" W. Alt. 2469m
Plantas rizomatosas, con rizomas cortos, erectas o ascendentes en la vegetación
Dupl.: LPB
10 marzo 2018
Martínez, E.J. 88
& Hojsgaard, D.H. & Glücksberg, A.
INCT 2015-1245
HERBARIO NACIONAL DE BOLIVIA
LPB
d
HERBARIO DEL INSTITUTO DE BOTANICA DEL NORDESTE (CTES)
Corrientes - Rep. Argentina
POACEAE
Paspalum volcanensis Zuloaga, Morrone & S.S. Denham
Det: Hojsgaard, D.
Bolivia, Tarija
Loc. Junacas. Cerca de Ruta Nacional 11. 21° 25' 27" S 64° 24' 27" W 2469 m.s.n.m.
Obs.
Leg. Martínez, E. J., Hojsgaard, D. & Glücksberg, A.
Fecha: 10/03/2018
N° 88
HgCl2

Figure 1. Processed and mounted plant materials of *Paspalum* species stored at two Herbaria. **a, b.** *P. procurrens* (Martínez et al. 91). **c, d.** *P. volcanense* (Martínez et al. 88). **a, c.** specimens at LPB. **b, d.** specimens at CTES.

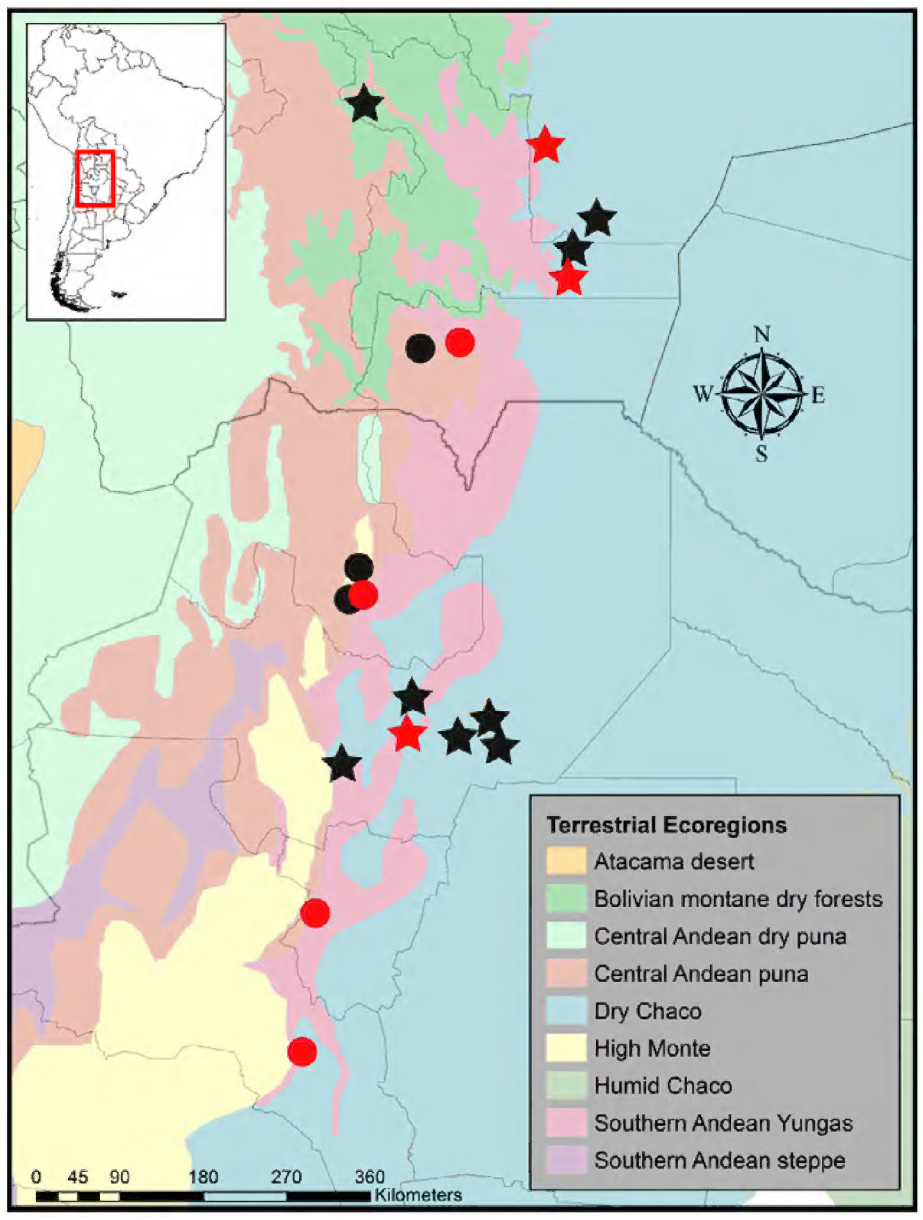

Figure 2. Geographic distribution of *Paspalum procurrens* and *P. volcanense* and associated terrestrial ecoregions in South America. Black circles represent previously known occurrences for *P. volcanense*, and red circles represent new records for this species and materials collected for the present work. Black stars represent previously known occurrences for *P. procurrens*, and red stars represent new records for this species and materials collected for the present work.

true in the departments of Tarija, Chuquisaca, and Santa Cruz from where some of the plant materials of our study were collected.

In the Dry Chaco ecoregion, large areas of the forest and open spots of natural meadows within the Chaco forest were transformed into croplands by the introduction of exotic C_4 grasses (Hoekstra et al. 2005). According to Volante et al. (2012), about 7.2% of the losses that have been suffered in natural habitats between 2000 and 2007 for northwestern Argentina has occurred in meadows and grasslands. In Argentina, especially in the ecoregion of the Dry Chaco, a large part of natural ecosystems have been lost due to the soybean crop (Grau et al. 2005).

In this study, we found new populations of both *P. procurrens* and *P. volcanense*. We also found a wider morphological variation compared to that previously reported for *P. volcanense* (Morrone et al. 2000; Zuloaga et al. 2000). Moreover, we found a mixed ploidy population of *P. procurrens*, with both diploid and tetraploid individuals. Although previous studies had identified populations of a single species of *Paspalum* subgen. *Anachyris* having individuals with different cytotypes growing together (i.e. in *P. simplex*, Urbani et al. 2002; Brugnoli et al. 2013), populations with mixed cytotypes are rare in nature. The coexistence of sympatric cytotypes is only possible under specific ecological conditions and particular biological features of the species (Kao 2007), and sympatric cytotypes are often associated to local adaptation mechanisms promoting intraspecific cytotype differentiation (e.g. through niche differentiation; Karunarathne et al. 2018). Ecological

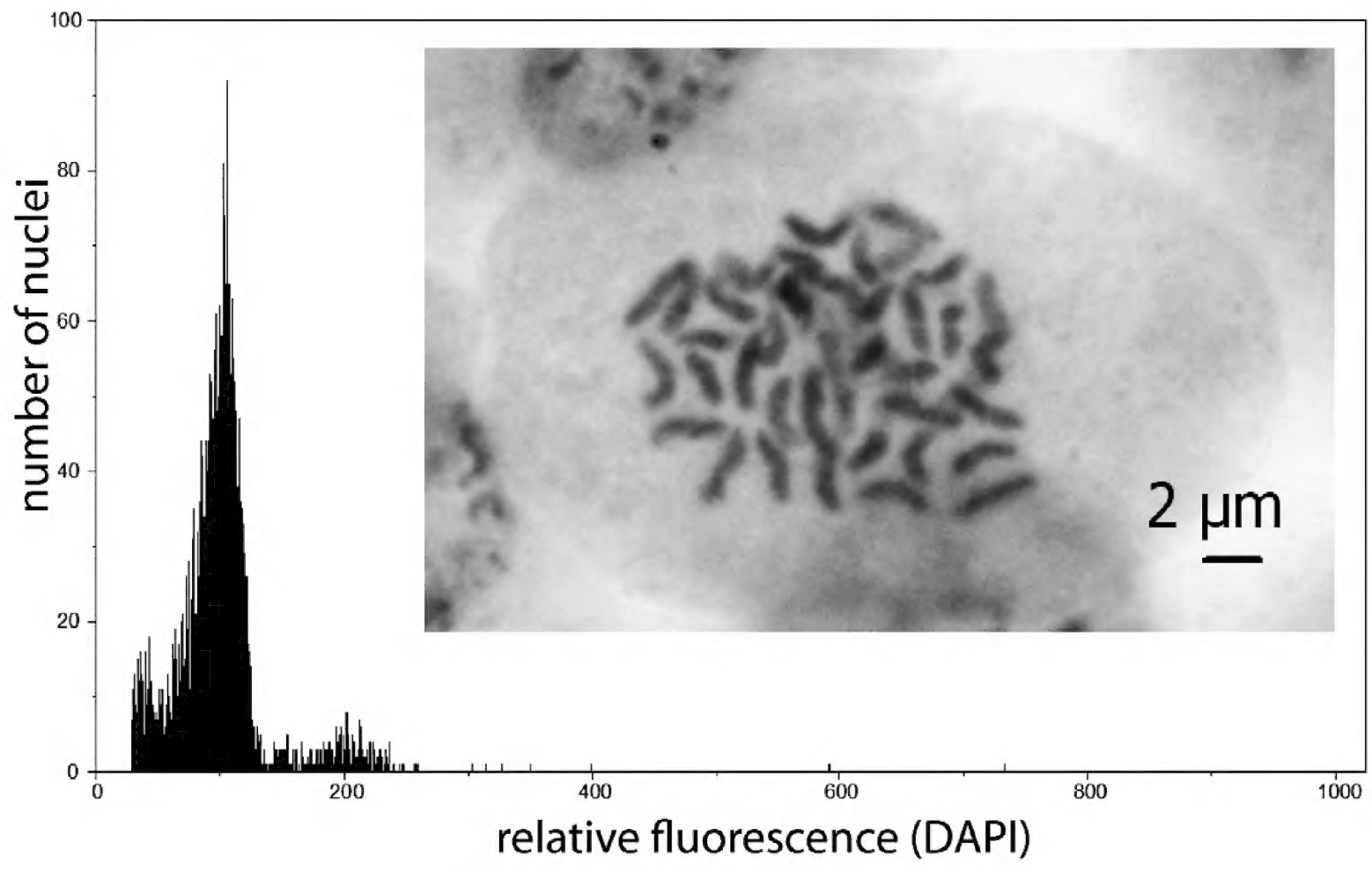

Figure 3. Analyses of ploidy levels in collected plant materials (Table 1). Flow Cytometry histogram of fresh leaf tissue (Martínez et al. 88) showing a high peak with a mean value around 100 in the *x*-axis corresponding with the relative position of the tetraploid standard material (Morrone 4374). The small peak around 200 in the *x*-axis corresponds to cells from Martínez et al. 88 in G_2 phase of the mitosis cell cycle. Image of a metaphase plate (Martínez et al. 88) showing 40 chromosomes.

differentiation among cytotypes growing in sympatry or in zones of contact frequently involves a differentiation in flowering time, which reduces hybridization among cytotypes and thereby facilitates their coexistence (Thompson 1992; Gross and Schiestl 2015). These processes enhance speciation events and therefore contribute to increased local diversity. Hence, the recognition of geographic distributions and intraspecific variability in species is important for better assessing potential effects of degradation on natural ecosystems and biodiversity. *Paspalum procurrens* and *P. volcanense* have not been sufficiently studied. Considering that the latest records for these species are more than 10 years old, and that habitats for these species are continuously exposed to modifications by humans, our new records are an important contribution towards the conservation of these taxa, the assessment of their genetic variability, and the understanding of the biodiversity.

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Author contributions

AG, EM, CM, and DH collected and prepared the specimens; DH made the taxonomic identifications; AG performed the Flow Cytometry evaluations; AG and DH carried out chromosome counts; AG and DH wrote the text with input from AH. All authors read and commented on the manuscript before submission.

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